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(54) Method, composition and device for measuring the ionic strength or specific gravity of a test sample.

(57) A method, composition and test device for determining the ionic strength or specific gravity of a test sample are disclosed. The method utilizes a test device comprising a test pad, wherein the test pad includes a carrier matrix incorporating a reagent composition capable of producing a detectable and measurable response that correlates to the ionic strength, and therefore the specific gravity, of the test sample. The reagent composition, comprising a strong polyelectrolyte, a metachromatic indicator dye and a suitable carrier, is incorporated into a carrier matrix to provide a test pad of a device useful in an ionic strength assay or a semiquantitative specific gravity assay of a test sample.

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FIELD OF THE INVENTION

The present invention relates to a method, composition and test device for determining the ionic strength or estimating the specific gravity of a test sample. More particularly, the present invention relates to a method of assaying an aqueous test sample, such as urine, for ionic strength or specific gravity that: a) utilizes a reagent composition that undergoes a detectable or measurable response upon contact of the test sample with the reagent composition, and b) is essentially independent of test sample pH. The detectable response is proportional to the ionic strength of the test sample and can be correlated quantitatively to the ionic strength of the test sample and semiquantitatively to the specific gravity of the test sample. The reagent composition provides sufficient color differentiation between test samples to provide an assay for the ionic strength or specific gravity of the test sample.

BACKGROUND OF THE INVENTION AND PRIOR ART

The specific gravity of a test sample, such as urine or serum, is a measure of the relative proportions of solid material dissolved in the test sample to the total volume of the test sample. In general, the specific gravity of a test sample is a measure of the relative degree of concentration or the relative degree of dilution of the test sample. The specific gravity of urine can be correlated to the ionic strength, or ion concentration, of the test sample. With regard to urine samples, the assay for specific gravity helps interpret the results of the other assays performed in a routine urinalysis.

Clinically, under appropriate and standardized conditions of fluid restriction or increased fluid intake, the specific gravity of a urine sample measures the concentrating and diluting abilities of the kidneys of an individual. The specific gravity of urine ranges from about 1.005 to about 1.030, and usually is in the range from about 1.010 to about 1.025. A specific gravity of about 1.025 or above in a random first morning urine specimen indicates a normal concentrating ability of the kidneys.

Either an abnormally low or an abnormally high urine specific gravity is clinically significant. Therefore, accurate and reliable specific gravity assays of urine and other aqueous test samples must be available for both laboratory and home use. The assays must provide an accurate measurement of abnormally low and abnormally high specific gravities, such that a correct diagnosis can be made and correct medical treatment implemented, monitored and maintained.

For example, diabetes insipidus, a disease caused by the absence of, or impairment to, the normal functioning of the antidiuretic hormone (ADH), is the most severe example of impaired kidney concentrating ability. This disease is characterized by excreting large urine volumes of low specific gravity. The urine specific gravity of individuals suffering diabetes insipidus usually ranges between 1.001 and 1.003. Low urine specific gravity also occurs in persons suffering from glomerulonephritis, pyelonephritis, and various other renal anomalies. In these cases, the kidney has lost its ability to concentrate the urine because of tubular damage.

An abnormally high urine specific gravity also is indicative of a diseased state. For example, the urine specific gravity is abnormally high in an individual suffering from diabetes mellitus, adrenal insufficiency, hepatic disease or congestive cardiac failure. Urine specific gravity likewise is elevated when an individual has lost an excessive amount of water, such as with sweating, fever, vomiting and diarrhea. In addition, abnormally high amounts of nonionic urinary constituents, like glucose and protein, increase the urine specific gravity to 1.050 or greater in some individuals suffering from diabetes mellitus or nephrosis. Urine with a fixed low specific gravity of approximately 1.010 that varies little from specimen to specimen is known as isothenuric. This condition is indicative of severe renal damage with disturbance of both the concentrating and diluting abilities of the kidney.

In order to determine if an individual has either an abnormally high or an abnormally low urine specific gravity, and in order to help monitor the course of a medical treatment to determine its effectiveness, simple, accurate and inexpensive specific gravity assays have been developed. In general, the specific gravity of a test sample is a measurement that relates to the density of the test sample. The specific gravity is a value derived from the ratio of the weight of a given volume of a test sample, such as urine, to the weight of the same volume of water under standardized conditions (Eq. 1).

$$Sp.\ Gr. = \frac{weight\ of\ urine}{weight\ of\ water} \qquad Eq.\ 1$$

Water has a specific gravity of 1.000. Since urine is a solution of minerals, salts, and organic compounds in water, the specific gravity of urine is greater than 1.000. The relative difference reflects the degree of concentration of the urine specimen and is a measure of the total solids in urine.

Several methods are available to determine the specific gravity of urine. The most widely used method, and possibly the least accurate, employs a urinometer. The urinometer is a weighted, bulb-shaped instrument having a cylindrical stem containing a scale calibrated in specific gravity readings. The urinometer is floated in a cylinder containing the urine sample, and the specific gravity of the urine is determined by the depth the urinometer sinks in the urine sample. The specific gravity value is read directly from the urinometer scale at the junction of the urine with the air. The urinometer method is cumbersome and suffers from the disadvantages of: a) requiring large volumes of urine test sample, b) a difficult and inaccurate reading of the urinometer scale, and c) unreliable assays because the urinometer is not regularly recalibrated.

Refractometry provides an indirect method of measuring the specific gravity of urine. The refractive index of urine is directly related to the number of dissolved particles in urine and, therefore, is directly related to the specific gravity of urine. Consequently, measurement of the refractive index of urine can be correlated to the specific gravity of urine. The refractometer method of determining urine specific gravity is desirable because specific gravity measurements are performed on as little as one drop of urine. However, the refractometer has the disadvantages of requiring daily calibration and not being amenable to home assays.

The falling drop method is another method of assaying for specific gravity which, like the urinometer, directly measures urine specific gravity. In this method, a drop of urine is introduced into each of a series of columns filled with solvent mixtures of increasing and known specific gravity. When the drop of urine comes to rest after its initial momentum has dissipated, and then neither rises nor falls, the specific gravity of the urine is determined to be identical to the specific gravity of the solvent mixture of that particular column. The falling drop method, however, is not widely used in routine urinalysis because of the lengthy time requirements in setting up such a assay and the inability of an individual to perform the assay at home.

The falling drop method described above also can be performed instrumentally. The instrument-based assay uses a specially designed column filled with a silicone oil having a controlled specific gravity and viscosity. The column is designed to measure the time required for a precisely measured drop of test sample to fall a distance defined by two optical gates (lamp-phototransistor pairs) mounted one above the other in a temperature-controlled column filled with a water-immiscible silicone oil of a slightly lower density than the test sample. The falling time is measured electronically and computed into specific gravity units. This specific gravity method is very precise, but the cost of the assay instrument and the degree of skill required to operate the instrument makes home testing for urine specific gravity impractical.

Not one of the above-described specific gravity assay methods is suited to performing specific gravity assays outside a medical office or laboratory. Consequently, reagent impregnated test strips were developed to enable an individual to perform specific gravity assays at home. In general, the test strip assay developed for specific gravity determinations is an indirect assay method, wherein the test strip changes color in response to the ionic strength of the urine sample. The ionic strength of a test sample is a measure of the type and amount of ions present in a test sample. The specific gravity of a test sample is proportional to test sample ionic strength. Therefore, by assaying for the ionic strength of a test sample, the specific gravity is determined indirectly and semiquantitatively by correlating the ionic strength of the test sample to the specific gravity of the test sample.

The present day specific gravity test strips are pH dependent, and comprise a carrier matrix impregnated with a reagent composition including a polyelectrolyte, such as a partially neutralized poly-(methyl vinyl ether/maleic acid); a chromogenic indicator, such as bromothymol blue; and suitable buffering agents. The reagent composition is sensitive to the number of ions, or electrolytes, in the test sample, such that the polyelectrolyte of the reagent composition undergoes an ion exchange, and releases hydrogen ions to the test sample in exchange for cations present in the test sample in an amount relative to the ionic strength of the urine sample.

Therefore, as the concentration of electrolytes in urine increases (high specific gravity), more cations are available to exchange with the hydrogen ions present on the polyelectrolyte of the reagent composition. The overall result is a release of hydrogen ions into the urine sample, and a resulting pH decrease of the urine sample that causes a color transition of the bromothymol blue chromogenic indicator from blue-green to green to yellow-green in response to increased specific gravity. The resulting color transition, indicating a pH change of the solution caused by increasing ionic strength, i.e., increasing specific gravity, is empirically and semiquantitatively related to the specific gravity of the urine sample.

For test strips utilizing the partially neutralized poly(methyl vinyl ether/maleic acid) polyelectrolyte and bromothymol blue indicator, assays for specific gravity are performed on aqueous test samples having a specific gravity of about 1.000 to about 1.030. A reading of 1.000, or a blue-green color, indicates that the urine has a very low specific gravity, as demonstrated by the lack of a color transition of the chromogenic indicator dye. A specific gravity reading of about 1.005 to about 1.030 is signified by color transitions, from blue-green through green to yellow-green, that serve as reliable indicators of increasing specific gravity.

It would be extremely advantageous to have a simple, trustworthy method of semiquantitatively assaying for urine specific gravity that allows visual differentiation of specific gravity values of about 1.000 to about 1.050. By providing a semiquantitative method of determining urine specific gravity in an easy to use form, such as a dip-and-read test strip, the urine assay can be performed by laboratory personnel to afford immediate test results. The specific gravity assay results can be interpreted in conjunction with assays for other urine constituents, such that a diagnosis can be made without having to wait for assay results and medical treatment can be commenced immediately. Furthermore, the test strip method can be performed by an individual at home to estimate the specific gravity of the urine and therefore to help monitor the success of the medical treatment the individual is undergoing.

As will be described more fully hereinafter, the method of the present invention is independent of test sample pH, and allows the fast and trustworthy assay for ionic strength, or specific gravity, of urine and other aqueous test samples by utilizing a test strip having a test pad that incorporates a reagent composition comprising a polyelectrolyte and an indicator dye capable of binding with the polyelectrolyte, like a metachromatic dye. The reagent composition undergoes a color transition in response to the ionic strength, or ion concentration, of the test sample. The color transition is directly related to the ionic strength of the test sample. Therefore, the reagent composition provides sufficient assay sensitivity to allow the quantitative determination of ionic strength and the semiquantitative determination of specific gravity.

Any method of assaying for the ionic strength or the specific gravity of urine or other aqueous test samples must yield trustworthy and reproducible results by utilizing a reagent composition that undergoes a color transition in response to the ionic strength or to the specific gravity of the test sample, and not as a result of a competing chemical or physical interaction, such as a pH change or preferential interaction with another test sample component, like protein or glucose. Additionally, the method and composition utilized in the ionic strength assay or specific gravity assay should not adversely affect or interfere with the other test reagent pads that are present on multiple test pad strips.

In accordance with the present invention, the reagent composition incorporated into the carrier matrix provides a sufficient sensitivity and color differentiation to assay for ionic strength, and therefore semiquantitatively assay for specific gravity. The method is especially useful for measuring test sample specific gravity from about 1.000 to about 1.015. In addition, although dry phase test strips have been used to assay for specific gravity, no dry phase test strip has incorporated a polyelectrolyte and an indicator dye capable of binding to the polyelectrolyte in an assay method for ionic strength or specific gravity of a test sample that is essentially independent of test sample pH.

Prior patents disclose the polyelectrolyte-dye ion exchange chemistry utilized in the above-discussed specific gravity assay of urine. For example, Falb et al. U.S. Patent No. 4,318,709 and Stiso et al. U.S. Patent No. 4,376,827 disclose a polyelectrolyte-dye technique used to assay for urine specific gravity. Each patent teaches utilizing polyelectrolyte-dye chemistry to determine the specific gravity of urine by monitoring the color transition of the dye.

The Falb et al. and Stiso et al. patents each disclose a composition and a method wherein the cations present in the test sample induce an ion exchange with the polyelectrolyte, thereby introducing hydrogen ions into the test sample. The change in hydrogen ion concentration, i.e., pH, is detected by a pH indicator. Accordingly, the previously disclosed methods are sensitive to the pH of the aqueous solution, and no direct interaction between the indicator dye and the polyelectrolyte occurs.

The composition and method of the present invention differ from the above disclosures in that a metachromatic indicator dye first binds to the polyelectrolyte. Then, upon contact between the present reagent composition and a test sample that includes cations, such as urine, the cations compete for available binding sites on the polyelectrolyte and displace a number of the metachromatic dye molecules from the polyelectrolyte. As will be discussed in more detail hereinafter, upon release from the polyelectrolyte, the spectral properties of the metachromatic dye molecules change and a color transition results. The color transition is directly proportional to the amount of metachromatic dye released from the polyelectrolyte, which in turn is directly related to the ionic strength of the test sample. The color change can be correlated, quantitatively, to the ionic strength of the test sample; and the ionic strength of the test sample can be correlated, semiquantitatively, to the specific gravity of the test sample. Accordingly, and in contrast to the Falb et al. and Stiso et al. disclosures, the present method is independent of test sample pH

because the color transition results from a pH-independent displacement of the metachromatic indicator dye from a polyelectrolyte, like a poly(vinyl sulfate).

It also should be understood that a pH indicator dye can be utilized in the method and composition of the present invention, as long as the pH indicator dye is capable of binding to the polyelectrolyte, and a buffer is included in the composition. The buffer ensures that the pH indicator dye changes color as a result of displacement from the polyelectrolyte, as opposed to either the pH of the test sample or a change in pH.

The present invention provides a composition and method for the accurate determination of ionic strength and the semiquantitative determination of specific gravity of urine and other aqueous test samples by utilizing a metachromatic indicator dye as the indicator component of a specific gravity reagent composition. European Patent Application 0 349 934 discloses a test strip and method of determining specific gravity or ionic strength of a sample utilizing a composition including a buffer, a complex former and a pH indicator dye. The complex former can be a crown ether, a cryptand, a podand or a multifunctional liquid. The method disclosed in the European Application is pH dependent, and utilizes a standard pH indicator dye, such as bromothymol blue or thymol blue. European Patent Application 0 349 934 does not teach or suggest a metachromatic dye or a polyelectrolyte utilized in the present invention.

Greyson et al. in U.S. Patent No. 4,015,462 discloses a support matrix incorporating osmoticallyfriable microcapsules containing a fluid including a dye. A portion of the microcapsules bursts upon contact with a test sample of low osmolality. A resulting release of the dye-containing fluid causes a color transition that is correlated to the specific gravity. However, the difficult production of the microencapsulated-containing supporting matrix is a serious disadvantage of the Greyson et al. method.

In contrast to the prior art, and in contrast to the presently available commercial test strips, the method of the present invention provides a sensitive measurement of test sample ionic strength, and provides a semiquantitative measurement of urine specific gravity, by utilizing a reagent composition including a metachromatic indicator dye, such as thionin, and a polyelectrolyte, like a poly(vinyl sulfate) or a poly-(styrenesulfonate), wherein the method is essentially independent of test sample pH. The present reagent composition undergoes a sufficient color transition upon contact with a test sample to provide an accurate ionic strength assay or to provide a semiquantitative determination of specific gravity. Hence, new and unexpected results are achieved in the dry phase reagent strip assay of urine and other aqueous test samples for ionic strength or for specific gravity.

SUMMARY OF THE INVENTION

In brief, the present invention is directed to a new and improved method and composition for determining the ionic strength or estimating the specific gravity of an aqueous test sample, and especially the ionic strength or the specific gravity of a biological fluid, such as urine, perspiration, or serum. The method includes using a reagent composition capable of interacting with cations in a test sample to produce a detectable and measurable response that can be correlated to the ionic strength or the specific gravity of the test sample. The response is essentially independent of the pH of the test sample. For home use, the reagent composition produces a visually detectable response. For laboratory use, the reagent composition produces a response that is detectable visually or instrumentally.

The method is suitable for dry phase assays, wherein the reagent composition is incorporated into a carrier matrix to provide a test pad of a test device. The carrier matrix of the test pad comprises a bibulous porous material, like filter paper, or a nonbibulous porous material, like a glass fiber or a permeable layer of a polymeric material. The reagent composition is homogeneously incorporated into the carrier matrix, and the carrier matrix then holds the reagent composition homogeneously throughout the carrier matrix in a known concentration while maintaining carrier matrix penetrability for the liquid test sample.

More particularly, the present invention is directed to a method of assaying for the ionic strength or the specific gravity of urine and other biological or aqueous test samples by utilizing a new reagent composition. It has been demonstrated that employing a reagent composition including a metachromatic indicator dye and a polyelectrolyte provides sufficient sensitivity to test sample ionic strength, and a sufficient color differentiation between test samples of different specific gravity, to permit the accurate measurement of ionic strength or the semiquantitative measurement of specific gravity. Surprisingly, the present method is essentially independent of test sample pH, and therefore it is often unnecessary to include a buffer in the reagent composition to measure the ionic strength or specific gravity of the test sample.

In accordance with an important feature of the present invention, the specific gravity of aqueous test samples can be determined, semiquantitatively, between about 1.000 and about 1.050, and especially between about 1.000 and about 1.015. By including a metachromatic indicator dye and a polyelectrolyte in

the reagent composition of the present invention, the assays are essentially independent of test sample pH and often the presence of a buffer is obviated. Accordingly, an improved assay sensitivity to ionic strength is achieved by utilizing the present reagent composition. The present reagent composition, including a metachromatic indicator dye and a polyelectrolyte, allows an accurate measurement of ionic strength, or the semiquantitative measurement of specific gravity, of urine or other test samples.

Therefore, one aspect of the present invention to provide a new method and composition for determining the ionic strength or the specific gravity of an aqueous liquid. The new composition interacts with cations in an aqueous test sample to produce a visible change, such as a change in color of a test device, that is indicative of the ionic strength or the specific gravity of the test sample.

Another aspect of the present invention is to provide a method of assaying urine or other aqueous test samples having sufficient sensitivity and sufficient visual color resolution to allow differentiation between, and the measurement of, test sample ionic strengths.

Another aspect of the present invention is to provide a method of assaying urine or other aqueous test samples utilizing a reagent composition capable of interacting with cations present in urine or other aqueous test samples, and undergoing a detectable and measurable color transition, independent of test sample pH, to establish the ionic strength or estimate the specific gravity of the test sample.

Another aspect of the present invention is to provide a reagent composition that interacts with cations present in the test sample and undergoes a visually or instrumentally differentiable color transition to allow the semiquantitative determination of test sample specific gravity from about 1.000 to about 1.050, and especially about 1.000 to about 1.015.

Another aspect of the present invention is to provide a method of assaying for the ionic strength or the specific gravity of a liquid test sample by incorporating a reagent composition into a dry phase detection device, wherein the reagent composition comprises: (a) a metachromatic indicator dye, like thionin or astrazon orange; (b) a polyelectrolyte, like a poly(vinyl sulfate) or a poly(styrenesulfonate); and (c) a suitable carrier.

Still another aspect of the present invention is to provide a new and improved method of assaying for the ionic strength or the specific gravity of an aqueous test sample by utilizing a test device including a carrier matrix having incorporated therein a reagent composition capable of interacting with cations present in the test sample, wherein the carrier matrix comprises a bibulous matrix, like filter paper, or a nonbibulous matrix, like a glass fiber or a layer of a permeable polymeric material.

A further aspect of the present invention is to provide an improved dry phase test strip that incorporates a reagent composition comprising a metachromatic indicator dye and a polyelectrolyte into the carrier matrix, and thereby provide an essentially pH independent assay in response to the ionic strength or to the specific gravity of a test sample.

The above and other aspects and advantages and novel features of the present invention will become apparent from the following detailed description of the preferred embodiments.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a two dimensional color space plot of A* vs. B* illustrating the color transition of the reagent compositions of Examples 1 and 2 to varying concentrations of sodium chloride.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

In accordance with the method of the present invention, the assay of aqueous test samples for ionic strength, or for specific gravity, is accomplished by utilizing a reagent composition that includes a metachromatic indicator dye and a polyelectrolyte. By employing a reagent composition including a sufficient amount of a metachromatic indicator dye and of a polyelectrolyte, sufficient sensitivity and sufficient visual color differentiation between test samples of differing ionic strengths are achieved. Surprisingly, the method is essentially independent of test sample pH, and therefore a buffer often is not necessary in the reagent composition. Accordingly, the accurate, reproducible ionic strength assay of aqueous test samples, independent of test sample pH, is provided. The composition and method also can be used to semiquantitatively determine test sample specific gravity because specific gravity can be correlated to ionic strength. The sensitivity and color resolution to test sample ionic strengths and specific gravities afforded by the method of the present invention are especially useful in urine assays.

Present day commercial test strip assays effectively measure specific gravities between about 1.000 and about 1.050. Present day test strips are not capable of accurately assaying for ionic strength. However, the present composition and method allow an individual to test for ionic strength at home with a test strip.

The present invention also allows the semiquantitative assay of test sample specific gravity because specific gravity can be correlated to ionic strength. The semiquantitative assay for urine specific gravity is clinically important because the urine specific gravity assay is interpreted in conjunction with assays for other urine analytes to assist in diagnosing a diseased state. The present invention is especially useful in assaying a urine sample having a specific gravity of about 1.000 to about 1.015. For urine specific gravities within the relatively normal range of from about 1.010 to about 1.025, the method of the present invention still affords sufficient color differentiation and sufficient sensitivity to urine specific gravity. However, clinical benefits are realized in this normal specific gravity range by interpretation of the specific gravity assay in conjunction with urine assays for other analytes, such that all of the assays can provide information concerning an abnormal physiological state that must be investigated further.

It will become apparent that in addition to assaying urine, the method and composition of the present invention also can be used to determine the ionic strength or specific gravity of blood plasma and serum; and more generally, the ionic strength or specific gravity of many other physiological fluids, like perspiration, as well.

To achieve the full advantage of the present invention, the method and composition are employed in dry phase, test pad assays to determine the ionic strength or the specific gravity of urine or other aqueous test samples. A dry phase test strip, including a test pad comprising a carrier matrix incorporating a reagent composition of the present invention, allows the rapid quantitative ionic strength assay or semiquantitative specific gravity assay of urine by visual means.

In particular, the present invention allows determination of ionic strength, or specific gravity, of a test sample by a visual color change of a test pad on a test strip. The test strip includes a test pad comprising an inert carrier matrix incorporating a reagent composition comprising a sufficient amount of a metachromatic indicator dye and of a polyelectrolyte. The ionic strength is determined from the color transition of the reagent composition. Test sample specific gravity is determined by semiquantitatively correlating the ionic strength of the test sample to test sample specific gravity.

The present composition and method allow the rapid colorimetric determination of the ionic strength or the specific gravity of a test sample. Previous specific gravity assay methods employed indicator dyes that are sensitive to, and therefore measured, solution pH. The present method utilizes a metachromatic indicator dye and a polyelectrolyte, but is essentially independent of the pH normally encountered in urine samples, e.g., pH of about 3 to about 9, often in the absence of a buffer. However, if the metachromatic dye also can act as a pH indicator, i.e., changes color in response to a pH change, a buffer is included in the composition to ensure that the metachromatic dye does not change color as a result of either test sample pH or a change in test sample pH.

The pH indicator dyes conventionally used in specific gravity assays undergo color transitions due to a pH change in the solution resulting from an ion exchange between the polyelectrolyte and cations present in the test sample. The phenomena is fully described in Falb et al. U.S. Patent No. 4,318,709 and Stiso et al. U.S. Patent No. 4,376,827, wherein the various dyes, the polyelectrolytes and the buffers required to observe the pH change are disclosed. The Falb et al. and Stiso et al. patents basically describe the present day dry phase test strips employed to assay for the specific gravity of urine. These present day test strips generally include: (a) an indicator dye that normally undergoes a color transition in the neutral pH range of about 6 to about 8, such as bromothymol blue; (b) a partially neutralized polyelectrolyte; and (c) a buffer.

In accordance with the methods of Stiso et al. and Falb et al., as the ionic strength of the urine increases, hydrogen ions are released into the solution due to an ion exchange between the cations in the test sample and the polyelectrolyte. The overall result is a drop in pH of the solution, and the bromothymol blue indicator changes color from blue-green to green to yellow-green in response to the pH change caused by increasing ionic strength. The increase in ionic strength of an aqueous test sample is directly related to an increase in specific gravity; the color transition of the dye therefore is empirically related to specific gravity values. This present day method allows specific gravities to be determined to within about 0.005. The present day method suffers from the disadvantage of color transition instability, wherein the color transition fades over a time period of minutes. Accordingly, the accuracy of the results is technique dependent.

In accordance with the present method, assays for the ionic strength or the specific gravity of an aqueous test sample are determined by examining a dry phase test strip for a visual color change after the test strip contacts a test sample. The test strip comprises a test pad, said test pad including a carrier matrix incorporating a reagent composition comprising a polyelectrolyte and a metachromatic indicator dye. In contrast to previous methods which utilized dyes that sensed the bulk pH of the solution, the present method is essentially independent of normally-encountered pH values and the reagent composition typically is essentially free of a buffer.

The above-discussed Falb et al. and Stiso et al. patents disclose the determination of ionic strength by inducing changes in the pH of the solution by an ion exchange with a polyelectrolyte. This pH change is measured by a pH indicator dye. The methods disclosed by Stiso et al. and Falb et al. are sensitive to the pH of the aqueous solution; no direct interaction between the pH indicator dye and the polyelectrolyte occurs; and a high urine pH (i.e., above 6.5) must be adjusted to obtain an accurate specific gravity assay. The methods discussed by Stiso et al. and Falb et al. also are sensitive to normal fluctuations in test strip manufacturing. For example, variations in carrier materials and in drying conditions of the test strip, and lot-to-lot differences in a polyelectrolyte, each can influence the final surface pH of the test strip. Variances in the final surface pH of the test strip result in inaccurate ionic strength or specific gravity measurements because the test strip becomes either more or less sensitive to pH changes.

The present invention differs from the methods disclosed by Stiso et al. and Falb et al. in that the present method is pH insensitive within the normal urine pH range (e.g., 3 to 9). This pH independence eliminates the need for specific gravity corrections of urine samples having a high pH. The present pH independent method also avoids the above-described manufacturing problems associated with the present pH-dependent test strips. In accordance with the present invention, first a direct interaction (e.g., binding) between a metachromatic indicator dye and a polyelectrolyte occurs. Then, a portion of the metachromatic indicator dye molecules are released from the polyelectrolyte after contact with the test sample. The released dye molecules undergo a spectral shift and, accordingly, a color transition that is essentially independent of pH and that is directly proportional to test sample ionic strength occurs. The color transition also can be correlated to test sample specific gravity to provide a semiquantitative specific gravity assay. A metachromatic dye that changes color in response to a pH change can be utilized, as long as a buffer is present to prevent a color change due to pH effects and to allow a color change resulting from release of the metachromatic dye from the polyelectrolyte.

The publication, "Interaction Between Cationic Dyes and Polyelectrolytes", by V. Vitagliano, Chemical and Biological Applications of Relaxation Spectrometry, E. Wyn-Jones, ed., D. Reidel Publishing Co., Boston, MA (1975), pp. 437-466, describes the interaction between a cationic dye, like a metachromatic dye, and a polyelectrolyte having negatively-charged sites, like a poly(styrene-sulfonate). It is known that the interaction, or binding, between a cationic dye molecule and a negatively-charged site of the polyelectrolyte is influenced by the ionic strength of a solution, with the binding between the dye molecules and the polyelectrolyte decreasing as solution ionic strength increases. The present invention utilizes this property in a pH independent method and device to assay an aqueous test sample for ionic strength, and accordingly, specific gravity.

In particular, the present invention utilizes a reagent composition comprising a strong polyelectrolyte and at least one metachromatic indicator dye to achieve maximum sensitivity to test sample ionic strength or specific gravity, independent of test sample pH. The metachromatic dye binds to a negatively-charged site on the polyelectrolyte. This binding of the metachromatic dye to the polyelectrolyte induces a spectral shift in the metachromatic dye due to dye molecules being in proximity to one another.

In particular, dyes that exhibit metachromasia are included in the present reagent composition because metachromatic dyes undergo a color change upon binding to, or release from, either a natural or a synthetic polyelectrolyte. Metachromasia is common in dyes having an electric charge partially delocalized into the chromophore group of the dye. As the metachromatic dye molecules bind to the negatively-charged sites on the polyelectrolyte, the metachromatic dye molecules interact with one another, thereby inducing a change in the spectral properties of the metachromatic dye, said change being observable as a color transition.

Cations affect the binding of the metachromatic dye to the polyelectrolyte because the cations successfully compete with the metachromatic dye for the available negatively-charged sites on the polyelectrolyte. Therefore, as the ion concentration of a solution increases (i.e., ionic strength or specific gravity increases), a greater amount of the metachromatic dye is released from the polyelectrolyte because the cations preferentially bind to the polyelectrolyte at the expense of the metachromatic dye. The release of metachromatic dye molecules from the polyelectrolyte into the solution results in a color transition because the spectral properties of the metachromatic dye return to the normal solution state spectral properties of the unbound dye. Therefore, the amount of metachromatic dye released from the polyelectrolyte, as determined by the color transition, can be correlated to test sample ionic strength (quantitatively) or specific gravity (semiquantitatively).

The method of the present invention utilizes the color transition that occurs as a result of cations present in the test sample causing a release of a metachromatic dye bound to a polyelectrolyte. Including a metachromatic dye and a strong polyelectrolyte in the reagent composition allows the ionic strength of a test liquid to be accurately and reliably measured. The ionic strength can be correlated semiquantitatively to

test liquid specific gravity. In accordance with an important feature of the present invention, the release of the metachromatic dye from the polyelectrolyte by cations present in the test sample provides a differentiable color transition that can be correlated to test samples having a different ionic strength. Accordingly, a more accurate measurement of test sample ionic strength is achieved because of improved assay sensitivity and because of improved color resolution between test samples of different ionic strength.

Therefore, the reagent composition of the present invention comprises: (a) a metachromatic indicator dye, (b) a strong polyelectrolyte, and (c) a suitable carrier. The reagent composition is used in a method, such as in a dry phase test strip method, to assay a test sample, like urine, for ionic strength or specific gravity.

The indicator dye utilized in the present invention is a metachromatic dye. The metachromatic dyes are capable of binding to negatively-charged sites on naturally-occurring and synthetic polyelectrolytes. Therefore, the metachromatic indicator dye often is a cationic compound. The visual spectrum of such metachromatic dyes changes when a monomeric form of the metachromatic dye binds to a polyelectrolyte. Therefore, a solution including a metachromatic dye bound to a polyelectrolyte undergoes a color change when the metachromatic dye molecules are released from the polyelectrolyte. Cations release metachromatic dye molecules bound to the polyelectrolyte because the cations preferentially compete with the metachromatic dye for the available negatively-charged sites on the polyelectrolyte.

In general, the metachromatic dye can be essentially any dye, preferably a cationic dye, that is capable of delocalizing a positive charge into the chromophore group. Such metachromatic dyes, after binding to a negatively-charged site of a polyelectrolyte, undergo a color transition in response to release from the polyelectrolyte due to the presence cations, like sodium or potassium. The degree and intensity of the color transition are directly related to the concentration of cations in the test sample; and the concentration of the cations is directly related to the ionic strength of the test sample. Therefore, the degree and intensity of the color transition can be correlated to the ionic strength, and in turn the specific gravity, of the test sample.

The particular metachromatic dye selected as the indicator dye component of the reagent composition can be determined by those skilled in the art of designing test kits in order to produce a specific gravity assay having maximum visual color resolution and maximum sensitivity. A metachromatic indicator dye included in the present reagent composition can be prepared by methods well known to persons skilled in the art. Furthermore, several metachromatic indicator dye compounds useful in the method of the present invention are well known dyes that presently are available commercially and are used as pH indicators.

The metachromatic indicator dye is present in the reagent composition at a concentration of about 5 to about 100 mM (millimolar, or millimoles per liter), and preferably from about 10 to about 80 mM. To achieve the full advantage of the present invention, the metachromatic dye is present in the reagent composition at a concentration of about 15 to about 60 mM.

Examples of metachromatic dyes that bind to a polyelectrolyte, then are released from the polyelectrolyte due to the presence of cations to undergo a color change, include, but are not limited to, thionin, astrazon orange, astrazon blue, toluidine blue, methylene blue, acridine orange, pyronine-G, proflavine, azure A, phloxine B, cresyl violet, safranine O, neutral red, thioflavin T, fast red AL, methylene green, rhodamine B, rhodamine 6G, azure B, neutral red, indoine blue, brilliant cresyl blue, 4',6-diamidino-2-phenylindoledihydrochloride hydrate, acridine yellow, acriflavine, pyronin-Y, pyronin-B, meldola's blue, nile blue, nile red, new methylene blue, methyl violet, quinaldine red, pinacryptol yellow, pinacyanol bromide, pinacyanol chloride, 2-[4-(dimethylamino)styrl]-1-methylquinolium iodide, 2-[4-(dimethylamino)styrl]-1-methylpyridinium iodide, stains-all, or a triphenylmethane dye, such as methyl green, crystal violet, victoria blue, brilliant green, basic fuchsin, new fuchsin, ethyl violet, malachite green oxalate, and mixtures thereof. A metachromatic dye that also is sensitive to pH, and therefore can be used as a pH indicator, is incorporated into the reagent composition in combination with a buffer to overcome the pH effects of the test sample, and allow the dye to undergo a color transition in response to cations present in the test sample as opposed to a pH change.

The reagent composition also includes about 0.2 to about 250 mM, and preferably about 0.5 to about 100 mM of a polyelectrolyte, as negatively-charged monomeric subunits. The polyelectrolyte has a sufficient number of negatively-charged sites to bind to the metachromatic indicator dye. To achieve the full advantage of the present invention, the reagent composition includes about 1 to about 10 mM, as negatively-charged monomeric subunits, of a polyelectrolyte.

Preferably, the polyelectrolyte is a strong polyelectrolyte, such as for example, but not limited to, a poly(vinyl sulfate), a poly(vinyl sulfonate), a poly(styrenesulfonate), poly(2-acrylamido-2-methyl-1-propanesulfonic acid), and mixtures thereof. Such strong polyelectrolytes ionize essentially completely in aqueous solution, thereby providing a sufficient number of negatively-charged sites for binding to the metachromatic indicator dye.

The molecular weight of the polyelectrolyte is not particularly critical. The interaction between the metachromatic dye and the polyelectrolyte is related to the concentration of negatively-charged moieties present in the composition, not to the molecular weight of the polyelectrolyte. Therefore, the critical aspect of the polyelectrolyte concentration is the amount of negatively-charged monomeric subunits present in the reagent composition.

As previously described, in aqueous solution, a metachromatic dye binds to the strong polyelectrolyte to provide a solution having a color different from an aqueous solution including the monomeric metachromatic dye. If cations are present, the cations preferentially compete for the available negatively-charged binding sites on the polyelectrolyte, thereby releasing the metachromatic dye to the solution. The aqueous solution then undergoes a color transition proportional to the amount of metachromatic dye released from the polyelectrolyte. The color transition is correlated to the amount of cations in the solution (i.e., the ionic strength), and in turn to the specific gravity of the solution.

In accordance with an important feature of the present invention, the present method is essentially independent of pH, and especially over the pH range normally encountered in urine samples (e.g., a pH of about 3 to about 9) because the color transition is proportional to the amount of metachromatic dye released from the polyelectrolyte, which is dependent only on the concentration of cations in the solution. Therefore, because the method is pH independent, the reagent composition including a metachromatic dye and a polyelectrolyte typically does not require a buffer.

If a buffer optionally is included in the reagent composition, any of various types of buffers can be used in the reagent composition of the present invention to provide a desired pH. The buffer optionally is included to maintain the reagent composition at a substantially constant pH and therefore optimize the response of a metachromatic indicator dye to the ionic strength of the test sample. The buffer also ensures that a metachromatic dye changes color in response to cations present in the test sample, as opposed to pH effects.

The amount of optional buffer included in the reagent composition depends upon the nature of the metachromatic indicator dye in the reagent composition. The concentration of the buffer usually is 0 to about 600 mM, and preferably 0 to about 300 mM. The particular buffer used in the reagent composition depends upon, and varies with, the metachromatic indicator dye and polyelectrolyte included in the reagent composition. For optimum assay results, the pH of the reagent composition is maintained at a pH value in the range of about 3 to about 9, and preferably in the range of about 4 to about 8. To achieve the full advantage of the present invention, the buffer maintains the pH value of the reagent composition about 5 to about 7.5.

If the metachromatic dye is capable of changing color in response to a change in pH, i.e., can act as a pH indicator, the metachromatic dye is buffered to a pH range wherein the metachromatic dye does not change color in response to pH changes, and wherein the response to cation concentration of the test sample is optimized. This pH range is readily determined by a person skilled in the art of designing diagnostic tests.

Exemplary optional buffers include, but are not limited to, acetate; BICINE; phthalate, borate; trichloracetate; sulfosalicylate; phosphate; tartarate; citrate; succinate; maleic acid; 2,2-bis(hydroxymethyl)-2,2',2''-nitrilotriethanol; 3,3-dimethylglutaric acid; 3-N-morpholinopropanesulfonic acid (MOPS); malonic acid; 1,3-bis[tris(hydroxymethyl)methylamino]propane(Bis-TRIS); tris(hydroxymethyl)aminomethane (TRIS); tris-(hydroxymethyl)aminomethane-maleic acid (TRIS-maleate); tris(hydroxymethyl)aminomethane-malonic acid (TRIS-malonate); 3-N-(trishydroxymethyl)methylamino-2-hydroxypropanesulfonic acid (TAPSO); 2-([tris-(hydroxymethyl)methyl]amino)ethanesulfonic acid (TES); 1,4-piperazinebis(ethanesulfonic acid) (PIPES); 4-morpholinoethanesulfonic acid (MES); N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES); and other suitable buffers well known in the art, or combinations thereof.

In addition to the metachromatic indicator dye and the polyelectrolyte, other optional ingredients, in addition to the buffer, that do not materially alter the nature or the function of the essential ingredients, and that do not interfere with the assay for specific gravity or ionic strength, also can be included in the reagent composition. For example, the reagent composition optionally can include a compound to improve the wetting of the test pad of the test device by the test sample. This compound usually is an anionic surfactant or a nonionic surfactant. A nonionic surfactant, such as an octoxynol, a nonoxynol or an ethoxylated fatty alcohol, is the preferred surfactant. An anionic surfactant, such as a long carbon chain sulfate or sulfonate, like sodium dodecyl sulfate, dioctyl sodium sulfosuccinate or sodium dodecylbenzenesulphonate, also can be included in the reagent composition of the present invention. The surfactant is included in the indicator reagent composition in a concentration of 0 to about 200 mM, and preferably in a concentration of 50 to about 200 mM.

The reagent composition also can include a polymeric material that improves the stability and uniformity of the color transition of the test device. Suitable polymeric materials include, but are not limited to, polyvinylpyrrolidone, polyvinyl alcohol, gum arabic, gelatin, algin, carrageenan, casein, albumin, methyl cellulose and similar natural and synthetic polymeric materials. The preferred polymeric material is a polyvinylpyrrolidone of average molecular weight 40,000 and available commercially from GAF Corp., New York, NY. The polymeric material generally is included in the reagent composition in an amount of 0% to about 5%, and preferably 0% to about 4%, by total weight of the reagent composition.

In addition, the reagent composition also can include a chelating agent in order to reduce the number of cations in solution capable of competing with the metachromatic dye for the negatively-charged sites on the polyelectrolyte. A more differential color transition between test samples having a high concentration of cations, i.e., electrolytes, therefore results.

The optional chelating agent utilized in the present invention is not particularly limited. However, an organic chelating agent, like a chelating dicarboxylic or polycarboxylic acid, or a polycarboxyalkylamine chelating agent, such as ethylenediaminetetraacetic acid, is most preferably employed. Other classes of useful chelating agents include, but are not limited to, a polyhydroxy compound, like sorbitol; a lignosulfonate; a glucoheptonate; dimethylglyoxime; salicylate complexes, like bissalicylaldehydeethylenediimine; dithionate derivatives; polyethyleneamines, like triethyleneamine; a 2,4-pentanedione derivative; a dipyridine derivative; triethylenepyridine amine; a polypeptide containing cysteine, glycine or histidine; a proline derivative; a thiocrown ether, like 1,4,8,11,22,25-octathiacyclooctosane; a triphenylphosphine; or combinations thereof.

Particular examples of chelating agents useful in the reagent composition of the present invention include, but are not limited to, tartaric acid, oxalic acid, malonic acid, succinic acid, citric acid, ethylenediaminetetraacetic acid (EDTA), gluconic acid, N-(hydroxyethyl)ethylenediaminetriacetic acid (HEDTA), nitrilotriacetic acid (NTA), diethylenetriaminepentaacetic acid (DTPA), aminotris(methylene phosphoric acid), hydroxyethylidene diphosphonic acid, hexamethylenediaminetetra(methylene phosphonate), ethylenediaminediacetic acid (EDDA), iminodiacetic acid (IDA), nitrilopropionic acid (NTP), hydroxyethyliminodiacetic acid (HIDA) and 1-hydroxyethane-1,1-diphosphonic acid; or combinations thereof.

The chelating agent can be added to the reagent composition in the free acid form, or in the form of a water-soluble salt, such as the sodium, potassium, lithium, ammonium, alkyl-substituted ammonium or hydroxyalkyl-substituted ammonium salt. The chelating agent is included in the reagent composition in a concentration of 0 to about 5 mM, and preferably of 0 to about 4 mM.

The carrier for the ingredients included in the reagent composition is water, a water miscible alcohol or a mixture thereof. Suitable alcohols are water-miscible alcohols, like, for example, but not limited to, methanol, ethanol, isopropyl alcohol and combinations thereof. However, because of the limited water or alcohol solubility of particular ingredients included in the indicator reagent composition, other organic solvents such as ethylene glycol, propylene glycol, acetone, dimethylformamide, dimethylsulfoxide, acetonitrile, ethyl acetate and similar solvents can be included in the carrier. The selection of a suitable organic solvent or solvents, in addition to water and alcohols, to include in the carrier of the reagent composition is within the capability of those skilled in the art of designing diagnostic assays.

The amount of organic solvent other than an alcohol present in the indicator reagent composition generally is 0% to about 50%, and preferably 0% to about 10%, by weight of the carrier. A carrier comprising water and an alcohol, like methanol or ethanol, is especially preferred because a carrier matrix impregnated with the reagent composition can be dried within a few to several minutes. In addition, the presence of an alcohol helps prevent precipitation of the metachromatic indicator dye by the polyelectrolyte.

As previously described, the reagent composition undergoes a color transition upon contact with a test sample to provide an assay for test sample ionic strength or test sample specific gravity. The intensity and degree of the color transition are used to quantitatively determine the ionic strength or to semiquantitatively determine the specific gravity of the test sample. In accordance with an important feature of the present invention, a reagent composition of the present invention provides a sufficiently resolved and differentiated color transition such that the ionic strength of a test sample can be measured and accurately determined without the use of color-measuring instruments, such as spectrophotometers or colorimeters. However, if desired, such color-measuring instruments can be used to measure the difference in color degree and intensity between the test sample and a solution having a known ionic strength. Because ionic strength is directly proportional to specific gravity, the color transition also can be correlated, semiquantitatively, to test sample specific gravity.

The intensity and degree of the color transition are used to determine the ionic strength or specific gravity of the test sample by comparing or correlating the color produced by the test sample to colors

produced by solutions having a known ionic strength or a known specific gravity. In accordance with an important feature of the present invention, the reagent composition provides a sufficiently resolved and differentiated color transition such that the ionic strength, or the specific gravity, of the test sample can be measured without the use of color-measuring instruments.

Accordingly, the pH-independent method of the present invention, utilizing a reagent composition including a metachromatic indicator dye and a polyelectrolyte, improves the accuracy and reliability of the ionic strength assay, and also increases physician confidence in the ionic strength assay. The method also provides a semiquantitative assay for specific gravity. Because of the large number of urine assays for specific gravity being performed at home by untrained individuals, as opposed to trained physicians or technicians in the laboratory, it is imperative to provide a fast and reliable semiquantitative assay method for the specific gravity of urine and serum that can be used in conjunction with assays for other urine constituents.

Conventionally, assays for specific gravity have been conducted at an essentially neutral pH using a pH indicator dye that undergoes a color transition at an essentially neutral pH in response to an ion exchange between cations in solution and the acidic hydrogen atoms of a polyelectrolyte. The pH indicator dye therefore is actually sensing the pH of the solution. In accordance with the method and composition of the present invention, test sample specific gravity is determined semiquantitatively by the color transition resulting from cations present in the test sample releasing a bound metachromatic indicator dye from a polyelectrolyte, which in turn causes a spectral shift in the metachromatic dye. The metachromatic indicator dye exhibits a different color spectrum in solution when bound to a polyelectrolyte, as opposed to the color spectrum exhibited when the metachromatic dye is in solution in the free state. Therefore, a color transition results when the metachromatic indicator dye is released from the polyelectrolyte due to the presence of cations in the test sample. Such a color transition is essentially independent of pH, because the color transition is related only to the color exhibited by the metachromatic indicator dye in its bound and free states. The degree and intensity of the color transition are directly related to the ionic strength, and therefore to the specific gravity, of the test sample.

To demonstrate the new and unexpected results achieved by the method and composition of the present invention, reagent compositions, including a strong polyelectrolyte and a metachromatic indicator dye, were prepared, then used in a dry phase assay for the specific gravity of a test sample. It has been demonstrated that a buffer often is not required in the reagent composition because the method is independent of test sample pH.

The aqueous solution of a metachromatic dye (toluidine blue) and a polyelectrolyte (potassium poly-(vinyl sulfate)) is red-violet in color and, after incorporation into a suitable carrier matrix, like filter paper, changes color ranging from red-violet to sky blue after contact and interaction with test samples having an increasing ionic strength or specific gravity. As a result, a reagent composition including a sufficient amount of a metachromatic indicator dye, like toluidine blue; and a strong polyelectrolyte, like potassium poly(vinyl sulfate) (KPVS), after incorporation into a suitable carrier matrix, produced the color transitions summarized in TABLE I upon contact and interaction with standard solutions including cations and having the following specific gravities:

TABLE I

COLOR TRANSITION OF A REAGENT COMPOSITION INCLUDING TOLUIDINE BLUE AND KPVS UPON CONTACT WITH STANDARDIZED SOLUTIONS		
Specific Gravity of Standardized Solution	Ionic Strength (molar)	Observed Color
1.000	0	red-violet
1.005	0.125	blue-purple
1.015	0.5	dull blue
1.025	1	sky blue
1.040	1.5	bright sky blue

In accordance with an important feature of the present invention, the color resolution achieved using the present reagent composition permits not only measurement of ionic strength, but also differentiation between test samples having different specific gravities.

To perform a dry phase, test strip assay for specific gravity, the reagent composition is produced first. For example, a reagent composition is produced by simply admixing the composition ingredients to provide an aqueous, alcoholic, or hydroalcoholic solution that is 5 mM in potassium poly(vinyl sulfate), as negatively-charged monomeric subunits, and 0.75 mM in toluidine blue. Preferably, the solution is a hydroalcoholic solution, including at least about 50% by weight alcohol.

A reagent composition including a metachromatic indicator dye and a polyelectrolyte, as described above, can be used in dry phase, test pad assays for ionic strength or for specific gravity. A dry phase, test pad assay utilizing the reagent composition is performed in accordance with methods well known in the art. In general, the assay for ionic strength or specific gravity is performed by contacting the urine or other test sample with an analyte detection device that includes the reagent composition. The analyte detection device can be dipped into the test sample, or the test sample can be applied to the analyte detection device dropwise. The resulting change in color of the analyte detection device reveals the ionic strength, or specific gravity, of the test sample; and, if so designed, the resulting color transition can be compared to a standardized color chart to provide a measurement of the ionic strength or specific gravity of the urine or test sample.

Typically, the analyte detection device is a test strip impregnated with a reagent composition, designed either as a single pad test strip (to assay only for a single analyte) or as a multiple pad test strip (to assay for several analytes simultaneously). For either type of test strip, the test strip includes a support strip, or handle, normally constructed from a hydrophobic plastic, and a reagent test pad, comprising a bibulous or nonbibulous carrier matrix. In general, the carrier matrix is an absorbent material that allows the test ample to move, in response to capillary forces, through the matrix to contact the reagent composition and produce a detectable and measurable color transition.

The carrier matrix can be any substance capable of incorporating the chemical reagents required to perform the assay of interest, as long as the carrier matrix is substantially inert with respect to the chemical reagents and does not contaminate the urine or other test samples either by test sample extraction of components comprising the carrier matrix or by appreciably altering the urine or test sample in a way to make the subsequent assays inconclusive, inaccurate or doubtful. The carrier matrix also is porous or absorbent relative to the liquid test sample.

The expression "carrier matrix" refers either to bibulous or nonbibulous matrices that are insoluble in water and other physiological fluids and that maintain their structural integrity when exposed to water and other physiological fluids. Suitable bibulous matrices include filter paper, sponge materials, cellulose, wood, woven and nonwoven fabrics, and the like. Nonbibulous matrices include glass fiber, polymeric films, and microporous membranes. Other suitable carrier matrices include hydrophilic inorganic powders, such as silica gel, alumina, diatomaceous earth and the like; argillaceous substances; cloth; hydrophilic natural polymeric materials, particularly cellulosic material, like cellulose beads, and especially fiber-containing papers such as filter paper or chromatographic paper; synthetic or modified naturally-occurring polymers, such as cellulose acetate, polyvinyl chloride, polyacrylamide, polyacrylates, polyurethanes, crosslinked dextran, agarose, and other such crosslinked and noncrosslinked water-insoluble hydrophilic polymers. The carrier matrix can be of different chemical compositions or a mixture of chemical compositions. The matrix also can vary in regards to smoothness and roughness combined with hardness and softness. The handle usually is formed from hydrophobic materials such as cellulose acetate, polyethylene terephthalate, polycarbonate or polystyrene, and the carrier matrix is most advantageously constructed from filter paper or polymeric films.

To achieve the full advantage of the present invention, the reagent composition is incorporated into a suitable carrier matrix to provide a test pad, and the test pad is utilized in a dry phase test strip for the ionic strength or specific gravity assay of an aqueous test sample. The method of the present invention provides an economical, accurate and reliable assay of aqueous test samples that can be performed at home or in the laboratory. In addition, the method of the present invention allows the differentiation and measurement of test sample ionic strength or specific gravity, therefore making the specific gravity assay more useful clinically.

In accordance with the method of the present invention, to perform a dry phase, test strip assay for ionic strength or specific gravity, the aqueous, alcoholic, or hydroalcoholic reagent composition described above, including about 0.1 to about 0.7 mM of a metachromatic indicator dye, such as thionin or astrazon orange; and 1 to about 10 mM of a polyelectrolyte, as negatively-charged monomeric subunits, like a poly-(vinyl sulfonate), first is prepared. A bibulous matrix, such as filter paper, like WHATMAN CCP500 filter paper, available commercially from Whatman Ltd., Maidstone, Kent, U.K., then is saturated with the reagent composition including the metachromatic indicator dye and the polyelectrolyte either by spreading, by immersing or by spraying the reagent composition onto precut strips of the filter paper. After removing the

aqueous, alcoholic or hydroalcoholic carrier by oven drying in an air oven at about 50 °C for about 15 to 20 minutes, the filter paper incorporating the reagent composition is cut to an appropriate size, such as a pad having dimensions of about 0.25 cm by about 0.25 cm to about 1 cm by about 1 cm. The filter paper incorporating the reagent composition then is secured to an opaque or transparent hydrophobic plastic handle with double sided adhesive tape.

The resulting test strip then was dipped into a fresh, uncentrifuged urine sample for a sufficient time to saturate the test pad with the sample. After waiting a predetermined time, such as about one minute to about two minutes, the test strip is examined, either visually or instrumentally, for a response. The degree and intensity of the color transition of the test pad reveal the ionic strength, or, if desired, the specific gravity, of the urine sample.

In accordance with another important feature of the present invention, it is well within the experimental techniques of those skilled in the art of preparing test devices to determine the proper balance between size of test pad; the strength of reagent composition; the identity and amount of the metachromatic indicator dye and the polyelectrolyte in the reagent composition; the amount of test sample; and the method of introducing the test sample to the test strip, such as by pipetting rather than dipping, to provide detectable and differentiable color transitions, such that a comparison, either visually or instrumentally, to color standards derived from solutions of known ionic strength or specific gravity is possible.

In many cases simple visual observation of the test strip provides the desired information. If more accurate information is required, a color chart bearing color spots corresponding to various standard ionic strengths or specific gravities can be prepared for the particular reagent composition used in the test strip. The resulting color of the test strip after contact with the urine sample then can be compared with the color spots on the chart to determine the ionic strength or specific gravity of the test sample.

If a still more accurate determination is required, a spectrophotometer or colorimeter can be used to more precisely determine the degree and intensity of the color transition. In addition, the dry phase, reagent strip assay can be made quantitative by employing spectrophotometric or colorimetric techniques, as opposed to visual techniques, in order to more reliably and more accurately measure the degree and intensity of the color transition, and therefore more accurately measure the ionic strength or specific gravity of the test sample.

To show the new and unexpected results achieved by using a reagent composition of the present invention in a method of determining the ionic strength or specific gravity of a test sample, color space plots were prepared from assays using dry phase test strips comprising a test pad incorporating a reagent composition including either a single metachromatic dye or a combination of metachromatic dyes, and a polyelectrolyte, into a filter paper matrix. The color space plots were obtained by contacting standardized solutions of known ionic strength or specific gravity with the dry phase test strips including the present regent composition incorporated into a filter paper carrier matrix.

In general, a color space plot includes three axes, the L*, A* and B* axes. The values of L* plotted on the vertical axis are a measure of the intensity of color, whereby a large L* value denotes a light color and L* = 0 denotes a completely black color. The horizontal A* axis is a measure of the color transition from green to red, whereby the more positive the A* value, the more red the color, and analogously, the more negative the A* value, the more green the color. Similarly, the third axis, B*, is a measure of the color transition from blue to yellow, whereby the greater the value of B*, the more yellow the color, and analogously the smaller the value of B*, the more blue the color.

The color space difference (ΔE) is calculated from the following equation (Eq. 2):

$$\Delta E = \sqrt{(L_1^* - L_2^*)^2 + (A_1^* - A_2^*)^2 + (B_1^* - B_2^*)^2} \qquad \text{Eq. 2}$$

wherein:

L_1^*, A_1^*, and B_1^* are the color space values determined for a first standardized solution of known specific gravity or ionic strength;

L_2^*, A_2^* and B_2^* are the color space values determined for a second standardized solution of known specific gravity or ionic strength having a different specific gravity or ionic strength from the first standardized solution; and

ΔE is the color space difference between the color space plots of the first and second standardized solutions.

The color space difference (ΔE) is the straight line distance between two points in a three-dimensional color space plot. Theoretically, a color space difference of one (1) unit is the smallest color space difference the human eye can distinguish. However, because of the inherent differences between the visual capabilities of individuals, a color space difference (ΔE) of about 3 units is required in order to practically and

confidently distinguish between colors.

The L*, A* and B* values plotted on the color space plots are calculated from the different reflectance measurements taken at sixteen different wavelengths evenly spaced between 400 nm (nanometers) and 700 nm using standard equations well-known in the art. In general, the percent reflectance at each of the sixteen different wavelengths is multiplied by the intensity of the light at that wavelength. These values then are multiplied by standard weighing functions for the colors red, green and blue, and finally added together. These calculations yield three tristimulus values, X, Y and Z. L*, A* and B* are calculated from the X, Y and Z tristimulus values using the following equations:

$$L^* = 116 \times [(Y/Y_0)^{1/3} - 16)] \quad \text{(Eq. 3)}$$

$$A^* = 500 \times [(X/X_0)^{1/3} - (Y/Y_0)^{1/3}] \quad \text{(Eq. 4)}$$

$$B^* = 200 \times [Y/Y_0)^{1/3} - (Z/Z_0)^{1/3}] \quad \text{(Eq. 5)}$$

wherein:

X_0, Y_0 and Z_0 are the tristimulus values for perfect white (i.e., reflectance = 100% at all wavelengths), and X, Y and Z are the tristimulus values calculated as described above from the sixteen wavelengths between 400 and 700 nm.

From the color space plots, the color space differences (ΔE) were calculated, and are summarized and discussed in more detail hereinafter. In interpreting the data to be presented, a term such as ΔE (1.007-1.022) is the color space difference between specific gravity assays for standardized urine solutions having a specific gravity of 1.007 and 1.022. Similarly, the term ΔE (0-0.12) is the color space difference between assays of standardized solutions having a sodium chloride concentration of 0 mM and 0.12 mM respectively. The terms ΔE (0.12-0.5) and ΔE (0.5-3) are analogously defined.

To demonstrate the unexpected results provided by a reagent composition of the present invention, the following compositions of Examples 1 and 2 were prepared. Then two sets of test strips were prepared, and used to assay for specific gravity and ionic strength of a test sample. Both sets of test strips utilized filter paper (WHATMAN CCP500) as the carrier matrix of the test pad.

EXAMPLE 1

One-half milliliter of a 1.5% by weight aqueous solution of potassium poly(vinyl sulfate) (KPVS) was admixed with 2 milliliters (ml) of distilled water. The resulting aqueous solution of KPVS then was admixed with 15 ml of methanol. Aqueous solutions of the metachromatic dyes thionin and astrazon orange were added, individually, to the methanol-water solution of KPVS to provide a reagent composition having a concentration of 2.5 mM KPVS (as negatively-charged monomeric subunits), 0.6 mM thionin and 0.13 mM astrazon orange. The carrier of the reagent composition was 92% by weight methanol. The presence of a high percentage of methanol and the order of addition of the ingredients help prevent the precipitation of thionin by the KPVS.

EXAMPLE 2

The procedure utilized in Example 1 was repeated to provide a reagent composition having a concentration of 5 mM KPVS (as negatively-charged monomeric subunits) and 0.075 mM toluidine blue.

The compositions of Examples 1 and 2 are reagent compositions of the present invention. The compositions each were incorporated into filter paper (WHATMAN CCP500), and the filter paper including either the composition of Example 1 or the composition of Example 2 was dried by standard procedures. The composition of Example 1 includes two metachromatic dyes and has demonstrated an excellent sensitivity to test samples having a wide range of ionic strengths and specific gravities. The composition of Example 2, including one metachromatic dye, has demonstrated an excellent sensitivity to test samples having a relatively low ionic strength or specific gravity.

Individual test strips incorporating either a composition of Example 1 or a composition of Example 2 were dipped into standardized sodium chloride solutions having a concentration of 0, 0.125, 0.5 or 3 mM sodium chloride and into standardized urine solutions having a specific gravity of 1.007 or 1.022. The resulting color transition of each test strip was determined and converted into ΔE units by standard procedures known in the art. The ΔE units for these experiments are summarized in TABLE II.

TABLE II

ΔE DIFFERENCES FOR ASSAYS UTILIZING A COMPOSITION OF EXAMPLES 1 AND 2				
Example	NaCl Concentration (mM)			Urine Specific Gravity
	ΔE(0-0.125)	ΔE(0.125-0.5)	ΔE(0.5-3)	ΔE (1.007-1.022)
1	7	13	25	5
2	12	12	3	10

In accordance with the method and composition of the present invention, from TABLE II, by including a metachromatic dye and a polyelectrolyte in a reagent composition to assay for ionic strength or specific gravity, the color space differences are at or above the minimum human detectable limit of approximately three ΔE units, thereby providing an ionic strength assay or specific gravity assay of the test sample. Generally, the color space difference values are at or above 3, therefore a color change is discernible by the human eye, and the assayer easily can differentiate between urine samples having different ionic strengths or specific gravities.

Specifically, a test strip including the composition of Example 1, incorporating a reagent composition including a combination of metachromatic dyes, showed an excellent sensitivity to sodium ions in the range of 0 to 0.125 mM that is perceptible to the human eye (ΔE = 7). The test strips also showed a sensitivity to sodium ions in the range of 0.125 to 0.5 mM (ΔE = 13) and in the range of 0.5 to 3 mM (ΔE = 25) that is readily perceptible to the human eye, thereby allowing an assayer to distinguish between test samples including 0.125 mM, 0.5 mM or 3 mM sodium ions. Similarly, an assayer can easily distinguish between a urine sample having a specific gravity of 1.007 and a sample having a specific gravity of 1.022 because the color space difference (ΔE) is a readily perceptible 5 units.

Test strips incorporating the composition of Example 2, including a single metachromatic dye, demonstrated an increased sensitivity to sodium ions present in a concentration of about 0.125 mM or less (ΔE = 12). However, sensitivity to sodium ion concentrations above about 0.125 mM is minimal, as demonstrated by the ΔE (0.125-0.5) value for sodium ion of 12, compared to the ΔE (0-0.125) value for sodium ion of 12. Because the ΔE values are equivalent, an assayer could not distinguish between a test sample that is 0.125 mM in sodium ion or is 0.5 mM in sodium ion. However, an assayer could detect and measure a sodium ion concentration below 0.125 mM because the color space difference exhibited is well above the minimum detectable level of 3 color space units.

It also should be noted that the ΔE (1.007-1.022) value of 10 in the specific gravity assay using a test strip incorporating the composition of Example 2 was greater than the ΔE (1.007-1.022) value (i.e., 5) using a test strip incorporating a composition of Example 1. This greater ΔE value translates into a more easily differentiable color transition and therefore a more sensitive gravity assay. The more differentiable color transition is attributed to weaker dye binding to the polyelectrolyte.

The results tabulated in TABLE II are illustrated in FIG. 1. FIG. 1 shows that the color transition for the reagent composition of Example 1 ranges from the red-yellow quadrant into the green-blue quadrant over the concentration range of 0 to 3 mM sodium chloride. The reagent composition of Example 2 undergoes a color transition from the blue-red quadrant into the blue-green quadrant over the concentration range of 0 to 3 mM sodium chloride. The color transitions for the reagent compositions of Examples 1 and 2 therefore are readily detectable, and differentiable, either visually or instrumentally.

As a result, using a metachromatic indicator dye and a polyelectrolyte in a reagent composition to differentiate and measure the ionic strength or the specific gravity of a test sample, allows the fast and reliable ionic strength or specific gravity determination of test samples. The present reagent compositions provide an important and useful benefit of providing a sensitive and accurate ionic strength assay and a semiquantitative specific gravity assay. As illustrated above, the metachromatic dye included in the present reagent composition responds directly to the cation concentration of the test sample, is essentially independent of pH, and provides a semiquantitative specific gravity or a quantitative ionic strength assay.

It should be understood that those skilled in the art of designing test kits are able to design an optimal test strip incorporating a sufficient amount of a particularly effective reagent composition to permit the differentiation and measurement of test sample ionic strengths and specific gravities. Because an assay utilizing the method and composition of the present invention exhibits a color space difference of at least 3 units. This ΔE value is sufficient for detection by the human eye, and is easily detected by present day colorimeters or spectrophotometers. Similarly, the method and composition of the present invention provide an accurate ionic strength assay or semiquantitative specific gravity assay regardless of varying amounts of

nonionic components, such as glucose or albumin, found in the test sample, as long as a sufficient number of cations are present in the test sample.

In accordance with another important feature of the present invention, full color development of a test strip including a metachromatic dye and a polyelectrolyte in the reagent composition occurs within about one-half minute to about two minutes after contacting the test strip with the test sample. Maximum color development occurs after about two minutes of contact. However, acceptable and trustworthy assay results are achieved when the test strip is examined for a color change about one-half minute after contact with the test sample. Such a short time for full color development of the test strip is an additional advantage of the reagent composition of the present invention. In addition, the color transition is sufficiently stable such that an accurate assay results from examining the test strip up to ten minutes after contacting the test sample. Therefore, test strips incorporating the reagent composition of the present invention can be used to obtain fast and more accurate ionic strength assays and semiquantitative specific gravity assays.

Overall, the metachromatic indicator dye and the polyelectrolyte included in a reagent composition incorporated into a suitable carrier matrix, such as filter paper, improves color differentiation between test samples having sufficiently different specific gravities, and provides excellent sensitivity to the ionic strength of aqueous test samples. In addition to excellent sensitivity, the method and composition of the present invention provide full color development and accurate assay results in a relatively short time.

Therefore, in accordance with an important feature of the present invention, more accurate and reliable assays ionic strength and semiquantitative assays for the specific gravity of urine and other liquid test samples can be performed by utilizing a metachromatic dye and a strong polyelectrolyte in a reagent composition. The metachromatic dye and polyelectrolyte improve the color differentiation between test samples having different ionic strengths and specific gravities, and are essentially independent of pH, thereby improving assay sensitivity.

Obviously, many modifications and variations of the invention as hereinbefore set forth can be made without departing from the spirit and scope thereof and therefore only such limitations should be imposed as are indicated by the appended claims.

Claims

1. A composition capable of exhibiting a detectable and measurable color transition in response to the ionic strength of an aqueous test sample, said composition comprising:
   (a) a sufficient amount of a metachromatic dye to provide a detectable color transition;
   (b) a polyelectrolyte capable of binding to the metachromatic dye; and
   (c) a carrier comprising water, a water miscible alcohol or a mixture thereof.

2. The composition of claim 1 further comprising: (d) a buffer.

3. A method of determining the ionic strength of an aqueous test sample comprising:
   (a) contacting the aqueous test sample with a reagent composition comprising:
      (i) a sufficient amount of a metachromatic dye to provide a detectable color transition;
      (ii) a polyelectrolyte capable of binding to the metachromatic dye; and
      (iii) a carrier comprising water, a water miscible alcohol or a mixture thereof, and
   (b) determining the ionic strength of the aqueous test sample from the intensity and degree of the color transition of the reagent composition.

4. The method of claim 3 wherein the reagent composition further comprises:
   (iv) a buffer.

5. The method of claim 3 wherein the aqueous test sample has a pH of about 3 to about 9.

6. A method of semiquantitatively determining the specific gravity of an aqueous solution comprising:
   (a) contacting the aqueous solution with a reagent composition comprising:
      (i) a sufficient amount of a metachromatic dye to provide a detectable color transition;
      (ii) a polyelectrolyte capable of binding to the metachromatic dye; and
      (iii) a carrier comprising water, a water miscible alcohol or a mixture thereof, and
   (b) determining the specific gravity of the aqueous solution from the intensity and degree of the color transition of the reagent composition.

7. The method of claim 6 wherein the reagent composition further comprises:
(iv) a buffer.

8. A method of determining the ionic strength of an aqueous sample comprising:
(a) contacting the aqueous sample with an analyte detection device comprising a test pad, said test pad having incorporated therein a reagent composition comprising:
(i) a sufficient amount of a metachromatic dye to provide a detectable color transition;
(ii) a polyelectrolyte capable of binding to the metachromatic dye; and
(iii) a carrier comprising water, a water miscible alcohol or a mixture thereof, and
(b) determining the ionic strength of the aqueous sample from the intensity and degree of the color transition of the reagent composition.

9. A method of semiquantitatively determining the specific gravity of an aqueous cation-containing sample comprising:
(a) contacting the aqueous sample with an analyte detection device comprising a test pad having incorporated therein:
(i) a sufficient amount of a metachromatic dye to provide a detectable color transition;
(ii) a polyelectrolyte capable of binding to the metachromatic dye; and
(iii) a carrier comprising water, a water miscible alcohol or a mixture thereof, and
(b) examining the analyte detection device for a color transition in response to the cation content of the aqueous sample; and
(c) correlating the color transition to the specific gravity of the aqueous sample.

10. An analyte detection device to determine the ionic strength of a aqueous test sample comprising:
a support strip;
a test pad; and
a reagent composition incorporated into the test pad, said reagent composition comprising:
(a) a sufficient amount of a metachromatic dye to provide a detectable color transition;
(b) a polyelectrolyte capable of binding to the metachromatic dye; and
(c) a carrier comprising water, a water miscible alcohol or a mixture thereof.

A* vs B* Color Space Plots for Examples 1 & 2

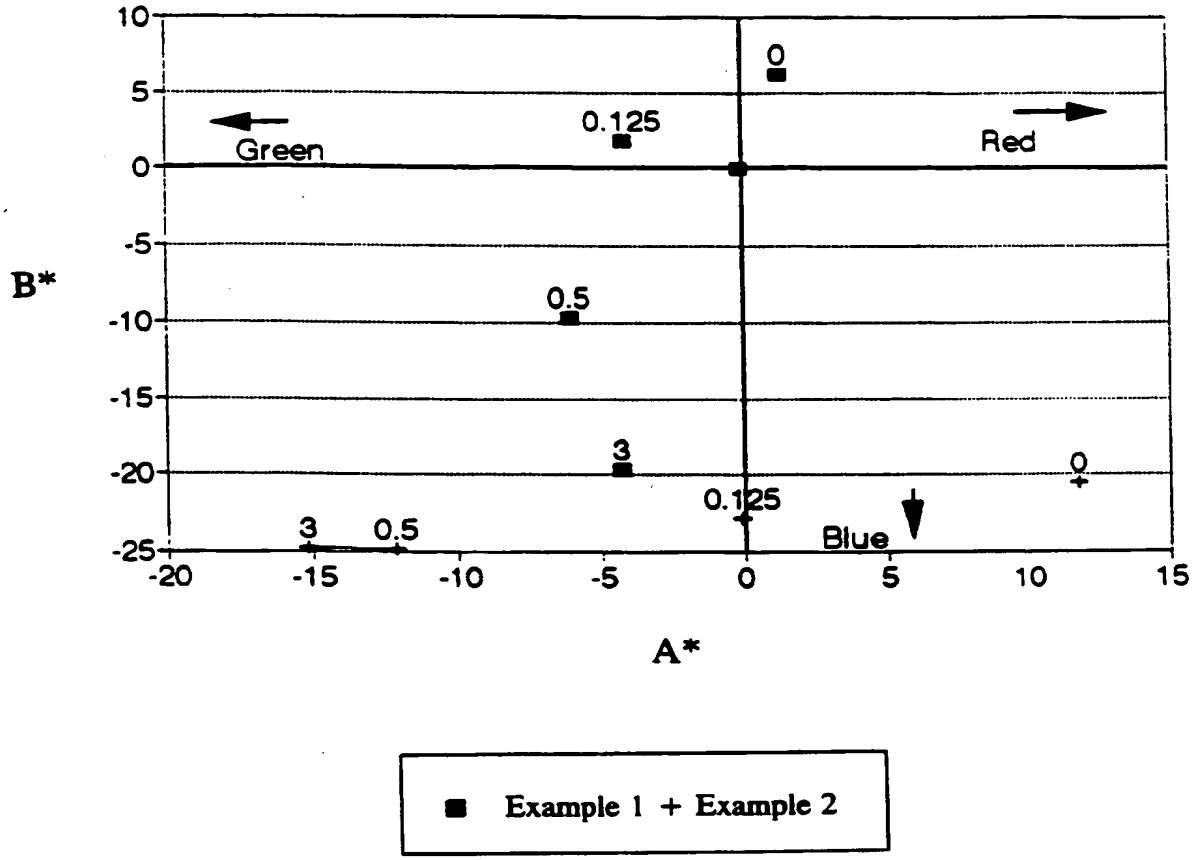

FIG. 1

European Patent Office

EUROPEAN SEARCH REPORT

Application Number
EP 94 10 1814

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.5)
X,D	US-A-4 376 827 (S.N.STISO ET AL.) * the whole document *	1-10	G01N33/52 G01N31/22 G01N9/00
X	EP-A-0 513 564 (BEHRINGWERKE AKTIENGESELLSCHAFT) * page 3, line 50 - page 4, line 39; claims *	1-10	
X,P	EP-A-0 580 863 (EIKEN KAGAKU KABUSHIKI KAISHA) * the whole document *	1-10	
A,P	EP-A-0 539 175 (WAKO PURE CHEMICAL INDUSTRIES LTD.) * claims *	1-10	
A	EP-A-0 114 316 (MILES LABORATORIES, INC.) * claims *	1-10	
A	EP-A-0 114 315 (MILES LABORATORIES, INC.) * claims *	1-10	
A,D	US-A-4 318 709 (R.D.FALB ET AL.) * claims *	1-10	
A,D	WYN-JONES 'Chemical and Biological Applications of Relaxation Spectrometry' 1975 , D.REIDEL PUBLISHING CO., , BOSTON, MA * page 437 - page 466 *	1	

TECHNICAL FIELDS SEARCHED (Int.Cl.5)
G01N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	7 June 1994	Hitchen, C

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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